Division of Laboratory Medicine

Wythenshawe Hospital

Laboratory Medicine Handbook

How to use this document:

Do not print it It may change, the most up-to-date version will always be available on the Trust internet/intranet

Click on items in this table of contents to navigate to the required section

- *Click to navigate between sections / open hyperlinks in a browser / email addresses to send a message*
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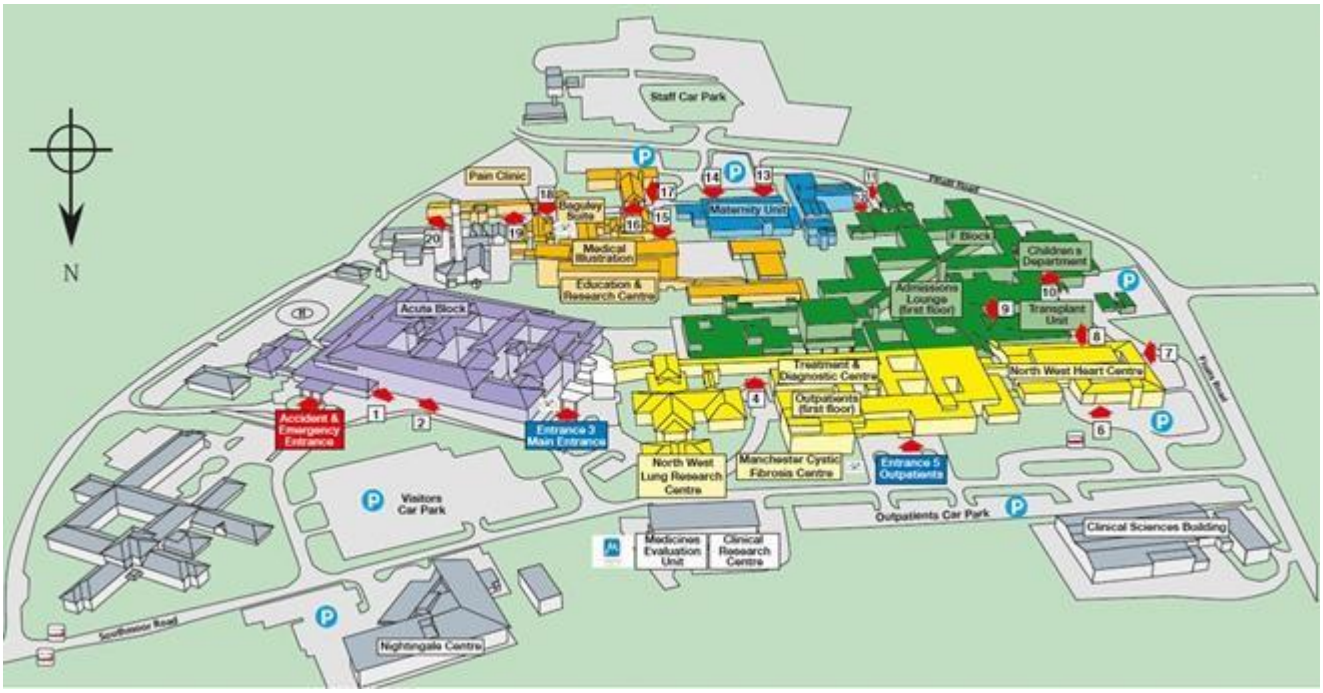
1 Introduction

The Division of Laboratory Medicine (DLM) at Manchester University NHS Foundation Trust (MFT) Wythenshawe Hospital provides diagnostic services to Wythenshawe and Withington Hospitals as well as General Practitioner surgeries in the South Manchester area and beyond. There are four departments within Division of Laboratory Medicine based at Wythenshawe Hospital; Biochemistry, Haematology, Cellular Pathology (including provision of mortuary services), and the Mycology Reference Centre Manchester (MRCM).

This handbook relates only to the Mycology DLM services located at Wythenshawe Hospital. Links to information for the other services can be found on the relevant pages in this handbook. Information on laboratory medicine services located at the Oxford Road Campus can be found here.

The Mycology Reference Centre is based in the Education and Research Centre, shown below, in orange.



1.1 Accreditation

Each laboratory within Laboratory Medicine has undergone external accreditation by the United Kingdom Accreditation Service, UKAS. Current accreditation status can be checked on the UKAS website, using the UKAS numbers in the below table. The accreditation process requires annual inspection to ensure there is a system of quality management in place to give assurance that the results released and advice given by the laboratory are of a high standard.

Laboratory	UKAS Number
Mycology	10196

1.2 Quality assurance

Tests performed in the laboratories are enrolled either in a national/international External Quality Assurance (EQA) scheme or a similar local scheme where no national scheme is available. This ensures that our tests are performing well. We also use Internal Quality Assurance (IQA) and Internal Quality Control (IQC) to monitor the performance of our tests.

Our clinical and some scientific staff are also enrolled on proficiency testing EQA schemes, where they are tested on their interpretation of case studies involving pathology test results. This ensures that the reports issued are correctly interpreted and the right advice is given.

Each laboratory is approved by the Institute of Biomedical Science for training of Biomedical Scientists, which has its own quality assurance programme.

The Biochemistry at Wythenshawe Hospital are recognised for training Specialty Trainees (STs) in each discipline. The training programmes are recognised and approved by the Royal College of Pathologists (and the General Medical Council) and are quality-assured by Health Education England North West. The Biochemistry department is an approved training centres for the Scientific Trainee Programme (STP), which is accredited by the National School of Healthcare Science.

As part of this training approval we have comprehensive training programmes for all grades of staff to ensure that they have all the required knowledge and skills to perform their duties to the highest level. This includes the provision of expert clinical advice to our users and interpretive comments on reports.

We also have Quality Managers within the laboratories who are employed to oversee all aspects of quality and ensure that we meet the requirements of our accrediting and regulatory bodies.

1.3 Key performance indicators

We use Key Performance Indicators (KPIs) to monitor the quality of the service we provide. There is a Laboratory Medicine dashboard that contains all of these KPIs, which is reviewed monthly to ensure we are performing to the required standard.

1.4 Protection of personal information

The laboratory adheres to the Trust-wide Policies on information governance for the protection of patient information, including the Trust “Data Protection Policy”.

1.5 Patient information

The Pathology Handbook is available to patients online.

1.6 Sample acceptance

All samples must have a request form. We require **four** patient identifiers on all samples and request forms. All patient identifiers must match exactly between the sample and the request form. These identifiers and further requirements are detailed in the table below.

Mandatory Labelling Requirement

Samples MUST be labelled with four unique identifiers which are as follows:

- District Number / NHS number
- Surname
- Forename
- Date of birth

Transfusion only – handwritten, signed and dated

If this information is not provided, no analysis will be performed. The event will be reported as an incident on Ulysses if appropriate.

Date and time of sample collection **Must** be provided to support sample validity

Multiple samples taken at different times on a patient **MUST** be labelled on the sample container with the time (24 hr. clock) when the sample is taken.

Electronic ordering must be used were available unless there is downtime, to reduce manual forms and associated transcription risks

The **request form (if required)** information **MUST** match the information on the **sample.**

Request forms MUST also contain:

- the patient's location/destination for the report (or a location code)
- Tests required
- Name of Consultant or GP
- Patient sex
- Date and time of sample collection
- Anatomical site and type of sample (where relevant)
- **All relevant clinical information**
  sample
- For Blood Transfusion – Form and sample **MUST** be signed by person collecting

If the information is not provided where the sample is repeatable/ reproducible, no analysis will be performed, and the sample will be discarded.

Where the sample is unrepeatable/ unreproducible, the risk to the patient of rejection of the sample must be weighed against the risk of acceptance of a wrongly labelled sample, local procedures will be followed.

Laboratory Medicine will accept no responsibility for samples analysed which initially failed to meet the acceptance criteria and will issue a disclaimer on such reports.

1.6.1 Samples failing acceptance criteria

If samples fail to meet the acceptance criteria they will not be processed. A report will be issued stating why the sample was rejected. Only unrepeatable samples will be processed, but **not before** the requestor has attended the relevant laboratory to complete a "Specimen Labelling Amendment Form". This form becomes part of the patient's record.

Examples of unrepeatable samples:

- Sterile body fluids (i.e. peritoneal, CSF, pleural)
- Bone marrow
- Fine needle aspirates (FNA)
- Tissue biopsies/surgical specimens/cytology samples (with the exception of sputum, urine and andrology samples)
- Blood cultures
- Mycology isolates from any of the above specimens
- Bronchoscopy/endoscopy specimens
- Drug levels timed for treatment (peak and trough), as well as any from neonatal patients
- Blood samples from patients with no venous access (i.e. femoral stabs)
- Arterial samples

All other samples will be rejected if they fail to meet the acceptance criteria. It is in everyone's interest, particularly the patients', that all samples and forms are correctly labelled before they are sent to the laboratory.

Please **do not** use addressograph labels on blood tubes or other small containers. These stickers are too large and the extra bulk they add means the samples will not pass through the automated analysers in the laboratories. Small labels can be used, except for transfusion samples.

1.6.2 Exceptions

Emergency departments (EDs) often care for patients unable or unwilling to give their identity including people who are unconscious or who have a critical illness, people with a mental health condition or delirium, and people affected by drink or drugs. The MFT Temporary Identification Criteria for Unknown or Unidentified patients Policy will be used to assign temporary identification to ensure the patient is uniquely identifiable and fulfils the Specimen acceptance policy minimal identification criteria.
In certain circumstances, patient identification details are intentionally hidden or substituted with particular ID numbers (e.g. Sexual Health, Clinical trials, donor samples) in such instances, a properly coded identifier must be used.

In all cases, the patient identification information on the sample must match that on the request form.

1.6.3 Patient consent

It is important that as well as the above, patient and family information is provided on the request form, where relevant (e.g. for interpreting genetic examination results). We may have to refer samples for testing to other laboratories if we are unable to perform the testing in-house. In these cases, we will have to send patient identifiers, such as name, date of birth and the clinical details we

have been given to enable these laboratories to perform appropriate testing and interpret results correctly.

The patient presenting to the point of sample collection or providing a sample themselves to their healthcare provider implies consent. The individual requesting the tests has responsibility for obtaining informed consent for these tests. Informed consent should cover all the tests requested, the implications of any results and that personal details and clinical information will be shared with the requesting organisation and any other organisations involved in providing the tests and results.

1.7 Requesting and reporting of laboratory tests

Electronic requesting must be used to request pathology investigations where it is available. Handwritten requests are more prone to sample labelling errors, transcription errors, sample collection errors and reporting errors. Do not use handwritten requests unless absolutely necessary.

Each request accepted by the laboratory for examination(s) shall be considered an agreement.

1.8 What to do when there is an IT failure

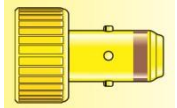
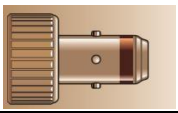
When there is a failure of the electronic requesting system and it is not possible to request pathology tests electronically, handwritten request forms can be used. Template downtime forms can be found on the Laboratory Medicine intranet pages.

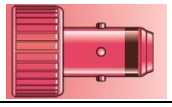
As part of the contingency process, all areas should store printed copies of the forms which can be photocopied as required. The laboratories will also hold a supply of printed forms for distribution if necessary.

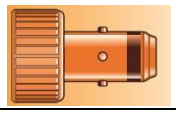
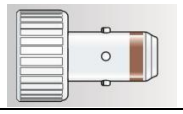
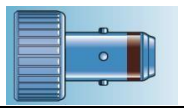
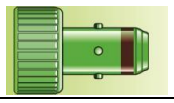
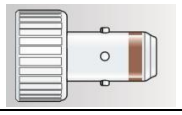
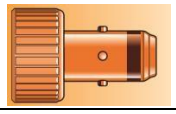
Critical/urgent results will be telephoned by Laboratory staff to the requesting departments. Please leave the Laboratory phone lines free for us to do this and do not contact us unnecessarily during these times.

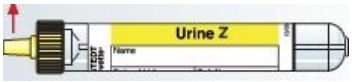
1.9 Sample containers

Sarstedt sample collection tubes are used for adults and paediatric patients. The lid colours are shown in the table below.

Container type		Colour	Description	Use
Fluoride		Yellow	Fluoride EDTA	Blood glucose Ethanol Lactate
Serum gel		Brown	Serum gel	Routine biochemistry Immunology HIT Screens **Please note these tubes MUST NOT be used for mycology tests. The gel may reduce the drug level detected.**

EDTA (3.4ml tube for adults, 1.8ml		Red	EDTA	Full Blood Count ESR Plasma Viscosity

Container type		Colour	Description	Use
for paediatrics and 1.2ml for neonates)				Haemoglobinopathy screens Malaria Red Cell Enzymes HbA1c Immunology Ammonia Immunosuppressants Cobalt & chromium
Li Hep		Orange	Lithium heparin gel	
Serum (no gel)		White	Serum (no gel)	Bacteriology Viral serology Immunology Mycology Some transfusion tests Fluid samples
EDTA		Blue	EDTA	Blood group Cross match **Please note these tubes MUST NOT be used for routine haematology tests**
Citrate		Green	Citrate	PT APTT Factor assays Anticoagulant monitoring Thrombophilia Screens D-Dimer Fibrinogen
Trace metal		White	Trace metal serum	• Zinc and Selenium
Rapid Lithium Hep Gel plus		Orange	Thrombinbased clot activator	• Only for routine biochemistry samples from A&E department
Paediatric tubes (1.2ml):				
Glucose fluoride		Yellow	Fluoride EDTA	Blood glucose Ethanol Lactate

Li Hep	 	Orange	Lithium Heparin	Routine biochemistry
EDTA 1.8 ml For Paediatrics 1.2 ml for neonatal		Red	EDTA	Full Blood Count ESR Plasma Viscosity Haemoglobinopathy screens Malaria Red Cell Enzymes
Container type		**Colour**	**Description**	**Use**
EDTA 1.2 ml for Neonatal 3.4 ml for paediatrics		Blue	EDTA	Blood Group Crossmatch DAT **Please note these tubes MUST NOT be used for routine haematology tests**
Serum gel		Brown	Lithium Heparin	Immunology **Please note these tubes MUST NOT be used for mycology tests**
Citrate		Green	Citrate	PT APTT Factor assays Anticoagulant monitoring D-Dimer Fibrinogen
Other sample containers:				
Urine tube		Yellow	Plain	Biochemistry Microbiology
Plain universal		White	Sterile container	Microbiology CSF samples for biochemical analysis CSF samples for mycological analysis Cytology samples

Plain universal with scoop		Blue	Sterile container with scoop for collecting faeces	Microbiology culture Biochemical analysis
24 hour urine collection				24 hour urine
• *Return to Examinations offered by biochemistry*				

1.10 Sample transportation

Each sample or set of samples must be placed in the plastic bag that accompanies the request form. This bag must be sealed to prevent any leakage or loss of samples in transit. Please do not use staples to seal these bags. Please ensure that the request sticker is placed on the paper form that is attached to the sample bag and NOT placed directly on to the plastic bag.

Samples must NOT be stored but should be sent to the laboratory immediately via porter or air tube or, for off-site users, by the next available transport. Users must consider the time of the next transport as delays may compromise certain results - if unsure, contact the relevant department.

1.10.1 Hospital requirements

Delivery in person to laboratory:

Samples must be sealed in plastic bags and must also be placed in an appropriate carrier, e.g. sturdy carry box, sealed strong bag or another approved container whilst being carried to the laboratory.

Pneumatic tube:

Samples in sealed bags may be placed directly into the pods and sent through the system. See below for more information on sample types that can and cannot be sent in a pod.

1.10.2 GP requirements

Samples collected from GP practices are gathered into strong polythene bags which are sealed. The hospital transport drivers place these bags in the secure rigid sample transport boxes with sealable lids that they carry in their vans.

These boxes must be labelled as “Diagnostic Specimens – UN3373” and have the department and hospital name and contact telephone number.

1.10.3 Postal samples

Samples that are sent via the Royal Mail must be packed in special containers purchased from the Royal Mail that conform to regulation UN No 3373 – Packing instructions for Diagnostic specimens and Infectious substances (Packing instruction P650). This states that the 'packaging must be of good quality, strong enough to withstand the shocks and loadings normally encountered during carriage'.

1.11 Requesting further tests on samples already in the laboratory ('add-on' tests)

Mycology Reference Centre

Please call us on ext. 2124 to request any additional tests, with patient identifiers. The addition of extra tests is dependent on sufficient sample volume and date of original sample collection.

1.12 Turnaround times

Turnaround times that appear in this handbook are from time of receipt into the laboratory information system (LIMS) to the time of reports being issued.

Where possible the time of receipt in the laboratory is recorded on the request form and corrected in the LIMS. This information is monitored monthly by each laboratory and is available on request.

1.13 Complaints or comments

We would hope that you do not have reason to complain about the service we provide. However, if you do, please use the contact details below or the specific departmental contact details to raise this with us in the first instance. Patient complaints can be made through the Trust website.

2 Phlebotomy

2.1 Inpatient service

A time limited service is provided to all medical and surgical wards Monday - Friday from between 8am and 12 noon for routine blood collection.

A restricted phlebotomy service is provided to the wards over the weekend and bank holidays to assist in the collection of routine blood samples between 7.30am and 11.30am.

All urgent blood requests must be taken by the doctor/nurse. Urgent requests should not be left for the phlebotomist's routine blood collection round.

2.2 Outpatient service

There is a drop-in phlebotomy service for out-patients. Please note this service is **not** for in-patient use.

Site	**Location**	**Days**	**Hours**
Wythenshawe Hospital	Phlebotomy suite opposite first floor Out-patient Department	Monday - Friday	09:00 am - 16:45 pm
Withington Community Hospital	Main Out-patients Department, ground floor	Monday - Thursday	08:30 am - 16:45 pm
		Friday	08:30 am - 16:00 pm

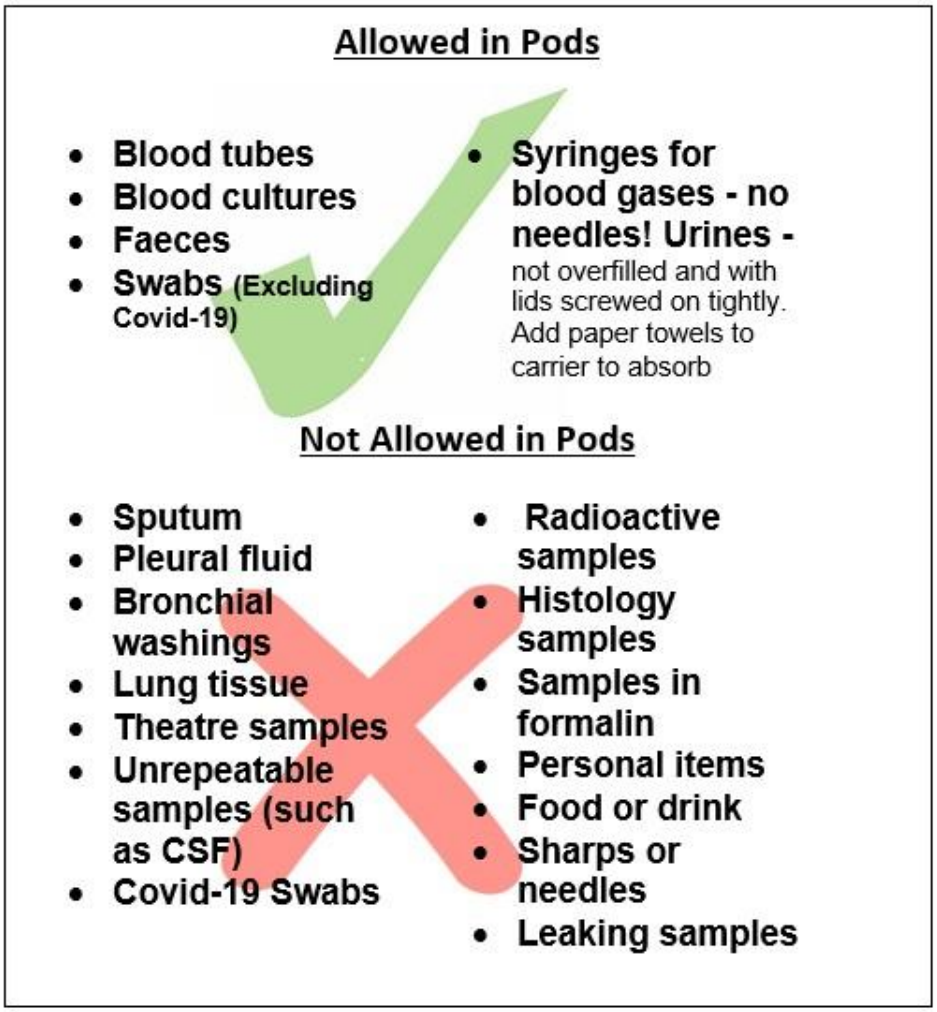
2.3 General Practitioner service

General Practitioner patients must use the appointment system and call 0800 092 4020 or 0161 947 0770 to book an appointment. **Please note -** patients must attend phlebotomy with a blood request form.

Site	**Location**	**Days**	**Hours**
Wythenshawe Hospital	Phlebotomy suite opposite first floor Out-patient Department	Monday - Friday	08:15am – 09:25am
Withington Community Hospital	Main Out-patients Department, ground floor	Monday - Friday	08:30 am – 12:25 pm
Burnage Health Centre	347 Burnage Ln, Manchester M19 1EW	Friday	09:00 am – 10.55 am

3 Sample reception

Pathology sample reception is open 24 hours a day, every day. Samples can be delivered directly to the hatch at specimen reception at the front of the Clinical Sciences Building or sent via the pneumatic tube. There are restrictions on samples that can be sent via the pneumatic tube, as detailed below:



This sign should be on every pod station. If your pod station does not have this sign please contact Sodexo on ext. 5430.

4 Cellular Pathology

For information on the Cellular Pathology service please click here.

5 Haematology

For information on the Haematology service please click here.

6 Immunology

For information on the Immunology service please click here.

7 Microbiology

The Microbiology Laboratory service for Wythenshawe Hospital is provided by the Manchester Medical Microbiology Partnership (MMMP), situated at the Oxford Road Campus. All information regarding the microbiology service is available on the MFT Laboratory Medicine website. Click on the MMMP User Manual link. The manual includes information on how to request samples out-of-hours and a list of contact details.

8 Biochemistry

For information on the Biochemistry service please click here.

9 Mycology Reference Centre, Manchester

The UK NHS Mycology Reference Centre Manchester (MRCM) is situated on the second floor of the Education & Research Centre at Wythenshawe Hospital. The MRCM provides specialist mycology diagnostic services for Manchester University NHS Foundation Trust, and hospitals throughout Greater Manchester and the UK.

Antifungal and mycological advice can be offered on the diagnosis of disease, clinical management and care of patients.

The MRCM laboratory is open from 08:30 to 17:00 hours Monday to Friday. Urgent medical advice can be obtained by contacting Wythenshawe Hospital switchboard and asking for the on-call Infectious Diseases Consultant.

9.1 Contact Details

Member of staff	Location	Extension	Email address
Dr R Richardson Clinical Lead and Head of Service	Office	5941	riina.richardson@mft.nhs.uk riina.richardson@nhs.net
	Secretary	5839	
	Mobile	07545 994 959	
Dr CB Moore Principal Clinical Scientist in Mycology and Deputy Head of Service	Office	4223	caroline.moore@mft.nhs.uk caroline.moore6@nhs.net
	Secretary	5839	
Professor MD Richardson Consultant Clinical Scientist in Mycology	Office	5914	malcolm.richardson@mft.nhs.uk
	Secretary	5839	
	Mobile	07545 994 936	
General enquiries	Office	5839	mrcm@mft.nhs.uk mft.mrcm@nhs.net
Test enquiries/results	Laboratory	2124	mrcm@mft.nhs.uk mft.mrcm@nhs.net
Website			www.mrcm.org.uk

All Wythenshawe Hospital samples should be sent to the Clinical Sciences Building, Wythenshawe Hospital where appropriate transportation to the Mycology Reference Centre Laboratory will be ensured.

External samples can be sent using the DX System. Our details are DX 332601 MANCHESTER 96M.

Alternatively, samples may be posted, ensuring appropriate packaging (see 1.10.3), to:
Mycology Reference Centre Manchester
2nd Floor Laboratories
Education and Research Centre
Wythenshawe Hospital
Southmoor Road
Manchester
M23 9LT

9.2 Additional Tests

Additional tests can be requested by contacting the laboratory, although it must be recognised that the archive sample available will have a limited volume. Furthermore, samples may be beyond the validation period for particular tests. Verbal requests for additional tests must be followed up in writing. The written request must include 3 patient identifiers, and the name of the requesting clinician.

9.3 Measurement Uncertainty

All assays have a margin of error associated with the calculation of the numerical value. This is referred to as the measurement uncertainty and is usually expressed as a percentage of the reported figure. This calculation allows the user to understand the uncertainty of any numerical results and can be assured with 95% confidence that the true result lies plus or minus the measurement uncertainty around the reported value. Further information on the measurement of uncertainty for our assays is available by contacting the laboratory.

9.4 Antifungal Drug Levels

Please ensure that details of **all** antifungal drugs the patient is receiving are given - this information is essential to ensure appropriate testing is performed.

Please record the date and time the specimen is taken, together with the time of last dose. These details ensure correct interpretation of results.

9.4.1 Indications for monitoring

- All patients receiving flucytosine
- Patients receiving itraconazole - to check drug absorption and to monitor compliance
- Patients receiving posaconazole - to check drug absorption and to monitor compliance
- Patients receiving isavuconazole – to check drug absorption and to monitor compliance
- All patients receiving voriconazole – a pre-dose sample is required
- Fluconazole in patients on dialysis/haemofiltration
- Patients failing azole therapy
- If drug interactions are suspected

9.4.2 References

- Richardson MD and Warnock DW. Fungal Infection: Diagnosis and Management. 4th Ed. Oxford, Wiley-Blackwell, 2012.
- Ashbee HR, Barnes RA, Johnson EM, Richardson MD, Gorton R, Hope WW. Therapeutic drug monitoring (TDM) of antifungal agents: guidelines from the British Society for Medical Mycology. J Antimicrob Chemother 2014; 69: 1162–1176.

9.5 Identification and susceptibility testing of medically important fungi

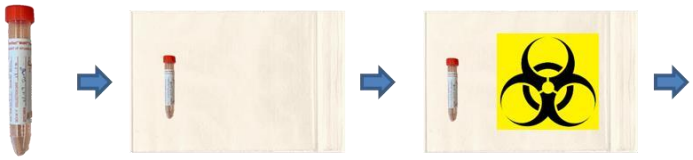
Genital, oral and wound swabs, along with respiratory and tissue specimens, will be cultured within our laboratory. Identification and/or susceptibility testing will be performed as appropriate.

9.5.1 Indications for testing

- All life-threatening fungal infections, to ensure that the optimal therapy is administered
- Isolates from patients at increased risk of fungal infection, such as those infected with HIV, immunosuppressed or on ICU, so that appropriate antifungal therapy can be given
- Mucosal candidosis not responding to therapy
- Clinically significant non-*Candida albicans* species due to increasing incidences of both infection and fluconazole resistance
- Rare pathogens because of an increased incidence of resistance and unpredictability of resistance patterns.

9.6 Sample collection and transportation

Sending respiratory samples from clinical areas:



Use double bags

Send to:

Central Specimen Reception (at ORC), Sample Reception (at Wyth) or Specimen Reception (at Trafford)

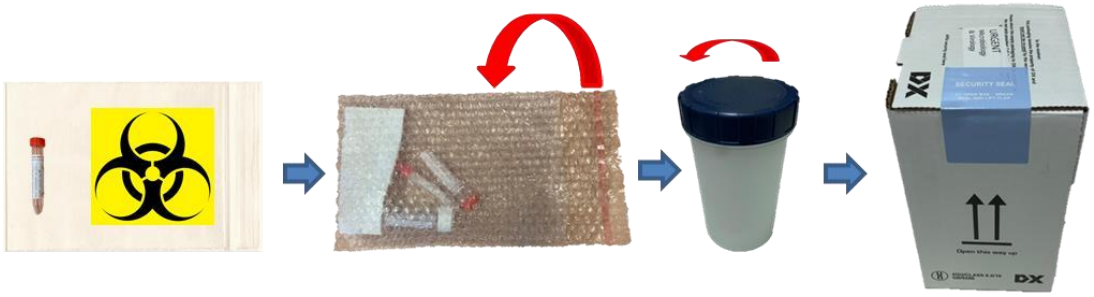
DO NOT USE AIR TUBE SYSTEM

- Samples should be placed into a plastic Ziploc bag and then into another plastic Ziploc bag (preferably with a biohazard label on).

- All respiratory samples for mycology testing (e.g. *Aspergillus* galactomannan) must be packaged in double bags as described above. **Do not submit samples with trap tubing still attached.** These samples are prone to leaking. The trap tubing **must** be replaced with a secure screw cap lid prior to placing in the specimen bag.

- Category B transport boxes are no longer required for transport of clinical specimens from clinical areas to sample reception on the same hospital site. Samples being transported by road, between hospital sites, **MUST** either be placed into a Category B transport box or an appropriate transport bag (i.e. one which adheres to regulations governing the transportation of diagnostic specimens.)

- Using the Cat B transport boxes:



Place single bagged sample into bubble wrap bag; seal and place into plastic cylinder; seal and place into cardboard box. Use supplied sticker to seal the box.

- Using the transport bags:

Place double bagged samples into the HCID transport bag. Multiple samples can be sent in the same bag.

9.7 Test library

Antifungal Drug Levels

Return to: Mycology Information Appendix A (list of tests)

Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
Antifungal drug levels		See below		Specimens should be transported to the laboratory as soon as possible. If a delay is anticipated, samples should be refrigerated. Assays validated for transportation of samples at room temperature for up to and including 5 days. **For all drugs, the time of previous dose and time of sampling should be recorded accurately to allow correct interpretation.** Gel separation tubes should **not** be used as the gel may reduce the drug level detected. More accurate results will be obtained by using tubes with no additive.
Flucytosine	Generally next working day	 Adult 4.9 ml Neonate 0.5 ml	Adult: Pre-dose 30-40 mg/L; Post-dose 70-80 mg/L Neonate: (<3 months) Pre-dose 20-40 mg/L; Post-dose 50-80 mg/L Levels >100 mg/L are potentially toxic	**Therapeutic Drug Monitoring is essential for clinical management** Pre-dose: Oral and IV: Just before dose* Post dose: • Oral: 2 hours post dose* • IV: 30 minutes post dose* Commence: Around second/third dose Frequency: Twice weekly, or more often if renal function is changing Lab assay runs: Day of receipt as required. Samples must be notified or received before 1pm

				Please inform the laboratory if patient is on any other antifungal, in addition to flucytosine, as this may affect test result. * these samples are most useful for clinical management

Antifungal Drug Levels

Return to: Mycology Information Appendix A (list of tests)

Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
Itraconazole	1-2 days	 Adult 4.9 ml Neonate 0.5 ml	• Target level: Prophylaxis: 0.5-1.0 mg/L Therapy: 1.0-2.0 mg/L • Commence monitoring only after steady state has been reached (1-2 weeks on oral therapy, little variation through the day)	**Therapeutic Drug Monitoring is essential for clinical management** Pre-dose: Oral: not needed Post dose: Oral: random* Commence: Only after steady state has been reached. Frequency: Dependent on patient - seek advice - usually monthly for the first three months and then every three months. Check levels two weeks after any dose change or if there might be a possible drug interaction. Repeat levels if concern about poor compliance / poor absorption. Lab assay runs: Each weekday * these samples are most useful for clinical management
Posaconazole	1-2 days		• Target level: Prophylaxis: 0.7-1.5 mg/L Therapy: 1.0-3.75 mg/L • Consider reduction if > 3.0 mg/L	**Therapeutic Drug Monitoring is essential for clinical management** Pre-dose: Oral: not needed Post dose: Oral: random* Commence: Only after steady state has been reached.

		 Adult 4.9 ml Neonate 0.5 ml	• Commence monitoring only after steady state has been reached (1-2 weeks on oral therapy, little variation through the day)	Frequency:	Dependent on patient - seek advice - usually monthly for the first three months and then every three months. Check levels a few weeks after any dose change or if there may be a drug interaction. Repeat levels if concern about poor compliance / poor absorption.
				Lab assay runs:	Each weekday
				* these samples are most useful for clinical management	

Antifungal Drug Levels

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Appendix A (list of tests)

Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information	
Voriconazole	1-2 days	 Adult 4.9 ml Neonate 0.5 ml	• **Pre-dose** level target range 1.3-5.7 mg/L • Dose escalation is advised for any level less than 1.3 mg/L • Due to the non-linear kinetics of the drug in adults, informed clinical judgement regarding target range is not possible on any sample except pre-dose samples	**Therapeutic Drug Monitoring is essential for clinical management**	
				Pre-dose:	Oral: 10-14 h post-dose window* (i.e. pre-dose as BD dosing) IV: Just before dose*
				Post dose:	Not required
				Commence:	after 3 days of therapy
				Frequency:	Dependent on patient - seek advice. If patient has suspected invasive disease or very unwell, do a level at day 5 of treatment and repeat at least weekly until therapeutic levels obtained. If IV to oral switch done, repeat level about 5 days after switch. Once therapeutic levels achieved, repeat levels at 2, 4, 8 and 12 weeks, and every three months thereafter. For other diagnoses, do a level at week 2, 4, 8 and 12 of treatment and every three months thereafter.

					Repeat levels two weeks after any dose change or if a drug interaction is suspected. Repeat levels if concern about poor compliance / poor absorption.
				Lab assay runs:	Each weekday

* these samples are most useful for clinical management

Antifungal Drug Levels

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information	
Isavuconazole	1-2 days	 Adult 4.9 ml Neonate 0.5 ml	• Target levels not fully established and informed clinical judgement is required for dose adjustments. Consult Mycology Reference Centre for advice. • On normal isavuconazole dosing, most patients achieve levels between 2.0 and 4.0 mg/L. • Levels above 5.0 mg/L have been associated with an increase in GI side effects. • The mean half-life of isavuconazole in plasma is 130 hours and it has linear kinetics.	**Therapeutic Drug Monitoring is essential for clinical management**	
				Pre-dose:	Oral: 10-24 hours post-dose window* (ie pre-dose as BD dosing) IV: Just before dose*
				Post dose:	Not required
				Commence:	after 3 days of therapy
				Frequency:	Dependent on patient - seek advice. If patient has suspected invasive disease or very unwell, do a level at day 5 of treatment and repeat at least weekly until therapeutic levels obtained. Once therapeutic levels achieved, repeat levels at 2, 4, 8 and 12 weeks, and every three months thereafter. For other diagnoses, do a level at week 2, 4, 8 and 12 of treatment and every three months thereafter.

					Repeat levels two weeks after any dose change or if a drug interaction is suspected. Repeat levels if concern about poor compliance / poor absorption.
				Lab assay runs:	Each weekday
				* these samples are most useful for clinical management	
Fluconazole levels can be assayed to test compliance or absorption. Contact the laboratory.					

Itraconazole, Posaconazole and Voriconazole TDM are performed by Department of Biochemistry, MFT Wythenshawe Hospital (UKAS 9063). Isavuconazole TDM is performed by Antimicrobial Reference Laboratory, Bristol (UKAS 8099). Check www.UKAS.com for up to date accreditation status of referral laboratories.

Fungal Culture

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information

Oral swabs, including mouth and throat swabs	Culture result (positive or negative) available by 5 days. See 'Identification and Susceptibility Testing of Yeasts and Moulds' below for further information.	Swabs:  Collect with liquid eSwab and transport in sealed plastic bags. Specimens other than swabs:  Collect into appropriate UKCA/CE-marked sterile leakproof containers and	N/A	Use aseptic technique. Collect specimens before antifungal therapy is started, where possible. Mouth swabs: To ensure that preconditions of sampling for oral infections are comparable, it is advised that patients should not: 1. Eat or drink within 2 hours 2. Brush their teeth within 2 hours 3. Use any mouth rinse of disinfectant within 2 hours prior to sampling If possible, samples should be taken in the morning under fasting conditions. Sample pus if present otherwise sample any lesions or inflamed areas. A tongue depressor or spatula may be helpful to aid vision and avoid contamination from other parts of the mouth. Throat swabs: Throat swabs should be taken from the tonsillar area and/or posterior pharynx avoiding the tongue and uvula. Liquid eSwabs contain 1ml of liquid. No liquid should be discarded when collecting sample. Samples with insufficient liquid may be discarded.

Fungal Culture

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information

		transport in sealed plastic bags.		Specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48 h are undesirable.
Genital tract swabs, including high vaginal swab (HVS), vaginal discharge, vulval swab, labial swab, cervical swab, endocervical swab, penile swab, urethral swab, and genital ulcer swab.	Culture result (positive or negative) available by 5 days. See 'Identification and Susceptibility Testing of Yeasts and Moulds' below for further information.	Swabs:  Collect into appropriate transport medium and transport in sealed plastic bags.	N/A	Use aseptic technique. Collect specimens before antifungal therapy is started, where possible. Cervical and high vaginal swabs should be taken with the aid of a speculum. It is important to avoid vulval contamination of the swab. High vaginal swabs: After the introduction of the speculum, the eSwab should be rolled firmly over the surface of the vaginal vault. Please use an eSwab and ensure the liquid remains in the tube. Cervical swabs: After introduction of the speculum to the vagina, the swab should be rotated inside the endocervix. Please use an eSwab and ensure the liquid remains in the tube. Urethral swabs: Contamination with micro-organisms from the vulva or the foreskin should be avoided. Thin swabs are available for collection of specimens. The patient should not have passed urine for at least one hour. For males, if a discharge is not apparent, attempts should be made to 'milk' exudate from the penis. The swab is gently passed

Fungal Culture

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
				through the urethral meatus and rotated. Place the thin swab in Amies transport medium with charcoal. Liquid eSwabs contain 1 ml of liquid. No liquid should be discarded when collecting sample. Samples with insufficient liquid may be discarded. Specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48hr are undesirable.
Wound swabs	Culture result (positive or negative) available by 5 days. See 'Identification and Susceptibility Testing of Yeasts and Moulds' below for further information.	Swabs:  Collect with liquid eSwab and transport in sealed plastic bags. Specimens other than swabs: 		Use aseptic technique. Collect specimens before antifungal therapy is started, where possible. Swabs for fungal culture should be taken using a liquid eSwab. Samples of pus/exudate, if present, are preferred to swabs. Sample a representative part of the lesion. Swabbing dry crusted areas is unlikely to yield the causative pathogen. If specimens are taken from ulcers, the debris on the ulcer should be removed and the ulcer should be cleaned with saline. If only a minute amount of pus or exudate is available, it is preferable to send a pus/exudate swab in transport medium to minimise the risk of desiccation during transport.

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
		Collect into appropriate UKCA/CE-marked sterile leakproof containers and transport in sealed plastic bags.		Specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48 h are undesirable.
Respiratory specimens, including bronchial aspirate, bronchoalveolar lavage, bronchial brushings, bronchial washings, endotracheal tube specimens, and expectorated or induced sputum.	Culture result (positive or negative) generally available by 14 days. Some specimens have extended culture up to 4 weeks. See 'Identification and Susceptibility Testing of Yeasts and Moulds' below for further information.	 Collect into appropriate UKCA/CE-marked sterile leakproof containers and transport in sealed plastic bags.	N/A	All specimens should be fresh and taken before antifungal treatment is started, where possible. Do not submit samples with Trap tubing still attached. These samples are prone to leaking and may be discarded. See Section 10.6 Sample collection and transportation for further details. Specimens should be transported and processed as soon as possible.

Tissue specimens	Culture result (positive or negative) available after extended culture of up to 4 weeks. See 'Identification and Susceptibility Testing of		N/A	Use aseptic technique. Collect specimens before antifungal therapy is started, where possible. Specimens received in formal-saline are not suitable for culture. If specimen is small, place it in sterile water to prevent desiccation.

Fungal Culture

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
	Yeasts and Moulds' below for further information.	 Collect tissue or biopsy material in appropriate UKCA/CE-marked sterile leakproof container **without** formalin and transport in sealed plastic bags.		Specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48 h are undesirable.

Identification and Susceptibility Testing of Yeasts and Moulds

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
Full identification and susceptibility testing of all medically important yeasts and moulds	All TAT are calculated from when the **culture** is available for testing. Identification: Yeast: 2-5 days Mould: 2-7 days Longer if molecular sequencebased identification is required. Susceptibility testing: Yeast: 1-5 days Longer for some yeasts and drugs. Mould: 3-7 days Longer for some moulds and drugs, or if culture requires prior incubation.	As per Microbiology guidelines for individual sample types	N/A	The following susceptibility tests are routinely performed: **Yeasts**: Flucytosine, Fluconazole, Amphotericin, Itraconazole, Voriconazole, Micafungin, Anidulafungin, Posaconazole **Moulds**: Itraconazole, Amphotericin, Voriconazole, Posaconazole, Isavuconazole, Micafungin, Terbinafine Other drugs, including caspofungin, are available upon request

Cryptococcal antigen lateral flow test

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
Lateral flow assay for cryptococcal antigen. Collection is not time dependent.	Generally same day	• CSF by lumbar puncture • Blood Minimum volume 500□l 	N/A	Indications for testing: • Testing for *Cryptococcus neoformans* capsular antigen is one of the most reliable methods for the diagnosis of cryptococcosis. • Suspected cryptococcosis, including cryptococcal meningitis, pulmonary and disseminated disease, in both immunocompromised, e.g. HIV-positive, and immunocompetent patients. With appropriate controls, a positive test is indicative of infection. Perform repeat lumbar puncture after 2 weeks of treatment. Repeated testing can be used to monitor response to treatment, monitor for duration of treatment course, especially in HIV-positive patients. <u>Limitations</u> The assay has not been evaluated for potential interference related to specimen pre-treatment with 2-mercaptoethanol, or with specimens including the following substances: vaginal cream, caffeine, ascorbic acid, itraconazole, amphotericin B, acetaminophen or acetylsalicylic acid.

Culture and identification of dermatophytes and non-dermatophytes from skin, nail and hair

Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
Culture	Direct microscopy: 1-4 days Culture and identification: 1-4 weeks. Longer for some unusual fungi.	Dermapaks, or similar envelopes, should be used. **Skin:** scrapings **Hair:** plucked hair roots and hair shaft **Nail:** nail clippings, scrapings of sub-ungual debris **Subcutaneous lesions:** scrapings, punch biopsies Adequate scrapings and clippings for direct microscopy and culture	N/A	No specific time of optimal collection, when patient presents with clinical presentation of superficial fungal infection and/or onychomycosis. Direct microscopy will be prioritised where there is insufficient material for full analysis.

Aspergillus galactomannan assay

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information

PLATELIA assay for *Aspergillus* galactomannan circulating antigen in serum and other body fluids: this test is indicated for presumptive diagnosis of *Aspergillus* infection	This assay is performed every day, Monday-Friday. TAT: 95% within one weekday.	 4.9ml clotted blood  Respiratory samples – minimum 2 ml: Collect into appropriate UKCA/CE-marked sterile leakproof containers and transport in sealed plastic bags.	The Platelia galactomannan (GM) test results are expressed as an index value and are reported as negative, weak positive, or positive – with the index value given. Interpretation of values depends on the sample type: **Blood**: GM index values of >0.5 are interpreted as positive. Recent data suggests that Invasive Aspergillosis (IA) needs to be considered if serum or plasma value is ≥0.7, however a single value <1.0 does not support the diagnosis of IA. Repeat testing is recommended if the disease is suspected. **Bronchoalveolar lavage fluid**: GM index values >1.0 are interpreted as positive. Index values 0.5-1.0 have a lower predictive value than values >1.0 and are interpreted as weakly positive. Further sampling is recommended. Recent data suggests that Invasive Aspergillosis (IA) needs to be considered if the index value is ≥0.8, however a single value <1.0 does not support the diagnosis of IA. Repeat testing is recommended if the disease is suspected. **Sputum**: The test is **not** validated for sputum samples. The cut-off value for this sample type has not	No specific time of optimal collection. First clinical indication of pulmonary or invasive aspergillosis. Prospective screening twice weekly to monitor for evidence of elevated and rising levels of galactomannan which provides a convenient surrogate marker for invasive or pulmonary *Aspergillus* disease (depending on sample type tested). This test should be used in conjunction with other diagnostic procedures. <u>Limitations</u> The performance of the Platelia *Aspergillus* Ag assay has not been evaluated with neonatal samples. There is a higher incidence in the number of false positive galactomannan results reported in European literature in samples from the neonatal population. Platelia *Aspergillus* Ag assay may exhibit reduced detection of galactomannan in patients with chronic granulomatous disease and Job's syndrome. Specific factors should be taken into account when interpreting the test: Galactofuranose has been demonstrated in various foods, particularly cereals, cereal products, and

Aspergillus galactomannan assay

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
			been established. In the presence of clinical suspicion, high values (>1) should trigger further investigations. Other respiratory samples can be processed but the test is **not** validated. Specificity of the test is improved if two or more consecutive specimens are positive.	cream desserts. Unlike human milk, cow's formulas frequently contain high concentrations of galactomannan. Dietary factors must therefore be taken into account in interpretation of the course of antigenemia in young children and more generally in all patients with an altered intestinal barrier. Positive test results in patients receiving piperacillin/tazobactam should be interpreted cautiously. In addition, semi-synthetic β-lactam treatments should be taken into account when interpreting the test. Administration of PLASMA-LYTE™ should be taken into account when interpreting results from serum and bronchoalveolar lavage fluid samples, since positive results have been observed in several studies.

Pan-fungal glucan assay

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information

FUNGITELL assay for (1-3)-D-glucan: the fungal glucan test is indicated for presumptive diagnosis of fungal infection	This assay is performed most days, Monday-Friday. TAT: 95% within two weekdays, generally available within one weekday.	 4.9 ml clotted blood **Samples are easily contaminated - it is recommended that blood is not separated before sending.**	The Fungitell test results are expressed in pg/ml of serum and range from undetectable (<31.25 pg/ml) to >500 pg/ml. Glucan values of <60 pg/ml are interpreted as negative results. Values ≥80 pg/ml are interpreted as positive. Values from 60 to 79 pg/ml are interpreted as indeterminate results and suggest possible fungal infection. Additional sampling is recommended. **The glucan test has a very high negative predictive value.** This is the possibility that patients with a negative screening test result do not have the disease (true negative). Test only validated for patients 18 years old and above.	No specific time of optimal collection. First clinical indication of invasive fungal infection. Prospective screening twice weekly to monitor for evidence of elevated and rising levels of glucan which provides a convenient surrogate marker for invasive fungal disease. This test should be used in conjunction with other diagnostic procedures. The Fungitell (1-3)-D Glucan assay does not detect certain fungal species such as the genus *Cryptococcus*, which produces very low levels of (13)-D-glucan. This assay also does not detect the Mucormycetes, such as *Lichtheimia*, *Mucor* and *Rhizopus*, which are not known to produce (1-3)-D-glucan. Limitations Positive results have been found in haemodialysis patients. Patients treated with certain fractionated blood products, such as serum albumin and immunoglobulins and in patients exposed to glucan containing gauze and surgical sponges, have been found to produce a positive result. Patients require 3-4 days for the restoration of baseline levels of serum 1-3-D glucan, after surgical exposure to 13-D glucan containing sponges and gauze. Interfering substances The following sample conditions can interfere with an accurate result:
Pan-fungal glucan assay				Return to: Mycology Information Appendix A (list of tests)

Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
				• Haemolysis • Sample turbidity caused by lipemia • The presence of visually apparent bilirubin • Turbid serum • Elevated levels of immunoglobulin G

Aspergillus PCR on respiratory samples

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
Elitech *Aspergillus* PCR	Seven days, performed twice weekly.	 Respiratory secretions (BAL, sputum) 1-2 ml minimum. Collect into appropriate UKCA/CE-marked sterile leakproof containers and transport in sealed plastic bags.	The Elitech *Aspergillus* PCR results are expressed as copies of *Aspergillus* spp. 18S rDNA; Ct values are also provided. Values of <120 copies are interpreted as negative results. Values >210 copies are interpreted as positive. Values from 120 to 210 copies are interpreted as indeterminate results and suggest possible fungal infection or colonisation.	No specific time of optimal collection. First clinical indication of pulmonary or invasive aspergillosis. False negative results may occur for a variety of reasons, for example inappropriate quality of sample and antifungal prophylaxis interference with assay. False positive results may occur if genera closely related to *Aspergillus* spp., e.g. *Penicillium* spp., are also present in the sample and detected due to the sequence similarity in the 18S rDNA. Assay for the quantitative detection of *Aspergillus* species genomic DNA extracted from respiratory specimens from the lower respiratory tract, as an aid to the diagnosis of pulmonary and invasive *Aspergillus* infection. The results need to be taken in context of the clinical condition of the patient and other diagnostic test results.

Aspergillus PCR on respiratory samples

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
				This test should be used in conjunction with other diagnostic procedures. Limitations Genomic DNA extracted from *Penicillium* spp, also generates positive results. Therefore, it must be noted that a positive result with this assay may be the result of infection by *Penicillium spp.,* rather than *Aspergillus* spp, Fungal culture may also be requested to aid diagnosis.

Aspergillus fumigatus cyp51A pyrosequencing

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information

Aspergillus fumigatus cyp51A pyrosequencing assay	Performed on demand – we aim for a TAT of 2 weeks. This may be up to 4 weeks, depending on the sample. .	 Respiratory secretions (BAL, sputum, 1-2 ml minimum). Collect into appropriate UKCA/CE-marked sterile	Sometimes DNA amplification fails, and no result is possible. Fifteen sites within the *cyp51A* gene of *Aspergillus fumigatus* are surveyed. Results are provided citing either no polymorphisms or resistance mutations in each site of *cyp51A* and an interpretation of the expected susceptibilities for itraconazole,	No specific time of optimal collection. Assay for the pyrosequencing-based detection of triazole resistance-associated polymorphisms of the *cyp51A* gene in *Aspergillus fumigatus*. All specimens are initially processed for *Aspergillus* PCR to determine suitability (>1000 copies 18S rDNA). The results need to be taken in context of the clinical condition of the patient and other diagnostic test results. Please contact MRCM for further information.

Aspergillus fumigatus cyp51A pyrosequencing

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
		leakproof containers and transport in sealed plastic bags. Culture of *Aspergillus fumigatus*.	voriconazole and posaconazole, according to published literature.	

Pneumocystis PCR

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
Pneumocystis PCR assay	4 days	 4.9 ml of EDTA blood	Threshold Cycle (CT) values are determined. Result is reported as positive or negative.	No specific time of optimal collection. First clinical indication of *Pneumocystis* infection. Specimens should be transported and processed as soon as possible. If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48 h are undesirable. False negative results may occur for a variety of reasons, for example inappropriate timing of sample collection, inappropriate sample, presence of virus below the

Pneumocystis PCR

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Appendix A (list of tests)

Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information

		 BAL, sputum - collect into appropriate UKCA/CE-marked sterile leakproof containers and transport in sealed plastic bags. 500 µl minimum. Sample daily if *Pneumocystis* infection is suspected.		detectable limit of the assay, antifungal prophylaxis interference with assay. New and emerging variants may also occur which may not be detected by this assay. If *Pneumocystis* levels in the sample are close to the limit of detection of the assay, sampling variation will result in lower reproducibility. This test is performed by Department of Virology, MFT Oxford Road (UKAS 8393).

Molecular identification of fungi from culture negative, microscopy positive specimens

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Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information

Pan-fungal PCR: DNA extraction and molecular sequencing	2-4 weeks. Performed on demand.	 Original specimen – collect into appropriate UKCA/CE-marked sterile leakproof containers and transport in sealed plastic bags. If a fluid specimen, such as pus, BAL, pleural fluid, peritoneal fluid, transport at room temperature in sealed plastic bags. Fixed paraffin blocks (**preferable**): transported in a sterile container at room temperature. Fixed paraffin sections: 10x normal (5 µm) or 5x thick (10 µm) consecutive sections placed together in a sterile container and transported at room temperature.	Fungal identification provided. Sometimes DNA extraction fails, and no result is possible.	Indications for testing: clinically significant fungal infection, with negative culture and serology. Using sophisticated DNA extraction technology, fungal DNA can be obtained from most samples in which fungal hyphae are seen, including fixed paraffin sections. In some cases, no sample was submitted for culture; in other cases, culture is negative. Cases should be discussed with the MRCM staff, who will advise. Sanger sequencing is provided by Eurofins Genomics GmbH.

Surveillance of hospital environments, homes, public buildings, for Aspergillus species and allergenic moulds

Test	TAT	Sample type	Biological Interval / Clinical Decision Values	Special precautions/ Information
Culture of air samples, culture of dust and material samples.	Initial results: 5-7 days Final report: 14 days	Air samples, settled dust, surface samples, material samples	N/A	No specific time. Surveillance during hospital construction, maintenance, demolition and renovation, water damage, faulty air filtration and conditioning, and outbreaks. Minimum: one air sample and one dust sample each from patients' rooms and general hospital areas. More intensive sampling where reservoir of *Aspergillus* is most likely to occur. Sampling availability, processing of submitted samples, identification, and interpretation: please contact the laboratory for further information.

10 Appendix A – A-Z List of tests

Test	Department
Antifungal Drug Levels	Mycology
Aspergillus fumigatus cyp51A pyrosequencing	Mycology
Aspergillus galactomannan	Mycology
Aspergillus PCR	Mycology
Aspergillus precipitin test	Mycology
Cryptococcal antigen lateral flow test	Mycology
Culture and identification of dermatophytes and non-dermatophytes from skin, nail and hair	Mycology
Fluconazole	Mycology
Flucytosine	Mycology
Fungal Culture	Mycology
Identification and Susceptibility Testing of Yeasts and Moulds	Mycology
Isavuconazole	Mycology
Itraconazole	Mycology
Molecular identification of fungi from culture negative, microscopy positive specimens	Mycology
Pan-fungal glucan assay	Mycology
Pneumocystis PCR	Mycology
Posaconazole	Mycology
Surveillance of hospital environments, homes, public buildings, for *Aspergillus* species and allergenic moulds	Mycology
Voriconazole	Mycology